CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
N-(((3,5-dichloro-2-fluoro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)amino)carbonyl)-2,6-diflurobenzamide

Chemical Code # 005816, Tolerance # 52905
SB 950 # NA
26 September 2002

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study on file[1]
Chronic toxicity, dog:	Data gap, no study on file[1]
Oncogenicity, rat:	Data gap, no study on file[1]
Oncogenicity, mouse:	Data gap, no study on file[1]
Reproduction, rat:	Data gap, no study on file[1]
Teratology, rat:	No data gap, no adverse effects
Teratology, rabbit:	No data gap, no adverse effects
Gene mutation:	No data gap, no adverse effects
Chromosome effects:	No data gap, no adverse effects
DNA damage:	No data gap, no adverse effects
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 186518 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T193920.wpd
P. Leung (10/31/02)

[1]This new active ingredient was submitted as a biochemical and these studies are not required at this time.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

No study on file.

CHRONIC TOXICITY, RAT

No study on file

CHRONIC TOXICITY, DOG

No study on file

ONCOGENICITY, RAT

No study on file

ONCOGENICITY, MOUSE

No study on file

REPRODUCTION, RAT

No study on file

TERATOLOGY, RAT

52905-011 186503, “XDE-007: Oral Gavage Developmental Toxicity Study in CD Rats”, (M.S. Marty and C.L. Zablotny, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 991040, 14 September 2000). 25 time-mated female CD rats received XDE-007 technical (98.6% purity) by gavage at 0 (0.5% methylcellulose), 250, 500, and 1000 mg/kg/day on gestation days 6 through 20. Implantation loss at the high dose was slightly increased relative to study controls but well within the range of historical control values. There were no affects on clinical signs, bodyweight, food consumption or organ weights (liver, kidney, uterus). **No developmental toxicity, no adverse effects. Maternal and Developmental NOEL = 1000 mg/kg/day. **Acceptable**. (Green and Gee, 10/4/02).

52905-009 186501, “XR-007: Whole Embryo Culture Teratogenicity Screen”, (E. W. Carney, Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, MI, Report # 971083, 31 July 1997). Seven non-pregnant female rats (serum donors) received 1000 mg/kg/day of the test article (XR-007, 98%) in 0.5% Methocel by gavage for 3 consecutive days. Four hours after the last dose, rats were exsanguinated and their blood centrifuged to obtain serum. Six control rats were similarly treated with vehicle and bled. Sera were heat inactivated (30 min. at 56°C), sterile filtered, and stored (-80°C) for 3 days. Rat conceptuses with intact amnion and visceral yolk sac, but with Reichert’s membrane removed, were explanted from a separate group of untreated, timed-mated rats on the afternoon of gestation day 9. Embryos were cultured in 100% sera from the control or treated donors, and, after 48 hours, evaluated for viability, growth, and morphology. All serum donors appeared normal throughout the test period.

Bodyweight gains were similar in control and treated animals. 12/12 treated and 11/12 control embryos had a beating heart and visible yolk sac circulation. Statistically significant increases in crown-rump length and somite number for treated embryos were reportedly due to a lower than usual growth rate in control embryos. Morphological abnormality was limited to an abnormal curvature of the anterior neural tube which distorted the head in one treated embryo (8.3%). No historical control data. This is **supplemental** information. (Green and Gee, 10/3/02).

TERATOLOGY, RABBIT

52905-010 186502, "XDE-007: Oral Gavage Developmental Toxicity Study in New Zealand White Rabbits", (E.W. Carney, *et al*., Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 991084, 15 March 2000). Twenty-five time-mated New Zealand White female rabbits received XDE-007 (98.6% purity) by gavage at 0 (0.5% methylcellulose), 250, 500, and 1000 mg/kg/day on gestation days 7 through 27. Absolute and relative maternal liver weights were slightly increased in treated groups relative to controls (not statistically significant). Implantation loss in treated groups was slightly higher than study controls but not statistically significant or dose-related. Fetal weight, live litter size, and incidence of malformations/variations were not affected. Maternal bodyweights, food consumption, and other parameters were comparable. **No adverse effects. Maternal and Developmental NOEL = 1000 mg/kg/day. **Acceptable**. (Green and Gee, 10/4/02).

GENE MUTATION

52905-013 186505, "*Salmonella - Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay Preincubation Method with a Confirmatory Assay with XDE-007". (Michael S. Mecchi, Covance Laboratories Inc., Vienna, VA., Covance Study # 22129-0-422OECD, Dow Study ID. 011001,** 20 June, amended 26 July and 14 August 2001). Triplicate cultures of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A were exposed to XDE-007 technical (98.4% purity), in the presence and absence of S9, at 0 (DMSO), 33.3, 100, 333, 1000, 3330, and 5000 *:*g/plate using the preincubation method. Cultures were treated, and preincubated for 20 ± 2 minutes at 37°C. Molten agar was then added, tubes were revortexed, and contents plated (petri dishes) and incubated for 52 ± 4 hours. Background lawn status and precipitation were evaluated. Positive controls were functional. No increase in the reversion frequency. **Acceptable.** (Green and Gee, 10/3/02).

52905-015 186507, "Evaluation of XDE-007 in the Chinese Hamster Ovary Cell/Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) Forward Mutation Assay", (V.A. Linscombe and D.J. Beuthin, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 011076, 26 July 2001). Chinese hamster ovary cells (CHO-K_1-BH_4) were exposed (4 hours) in duplicate cultures (1 x 10^6 cells/dish), in the presence and absence of S9, to XDE-007 technical (98.4% purity) at 0 (DMSO), 12.5, 25, 50, 100, and 200 *:*g/ml in the main assay and at 0, 6.66, 20, 66.6, and 200 *:*g/ml in the confirmatory assay. Cultures were trypsinized at the end of treatment and replated in duplicate at a density of 1 x 10^6 cells/100 mm dish for phenotypic expression (7 to 9 days). In addition, 200 cells/60 mm dish (3 dishes/replicate) were also plated and incubated 6-8 days to allow colony formation to determine toxicity. At the end of expression, cells were plated in 10 replicate dishes per initial concentration with 6-thioguanine for selection. Cloning efficiency was also determined. No increase in forward gene mutation at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus. Positive controls were functional. **Acceptable. (Green and Gee, 10/3/02).

CHROMOSOME EFFECTS

52905-009 186504, “Screening of XR-007 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes”, (V. Ann Linscombe, Health and Environmental Research Laboratories, The Dow Chemical Company, Midland, MI., Report # 971069, 16 June 1997). Lymphocytes from male Sprague-Dawley rats (100 :l of whole blood/ml of complete culture medium) were exposed in duplicate cultures to XR-007 at 0 (1% dimethylsulfoxide), 0.5, 1.67, 5.0, 16.7, 50, 167, and 500 :g/ml for 4 (+20 hours) and 24 hours in the presence and absence of S9 respectively. Treatment began approximately 48 hours after initiation of the cell cultures. Aberration results were reported for lymphocytes treated at 50, 167, and 500 :g/ml. These levels were chosen based on mitotic indices. An increase in clastogenic activity was not indicated. **Unacceptable**, upgradeable (description, justification, and discussion of assay performance and results; GLP). (Green and Gee, 10/3/02).

52905-016 186508, “Evaluation of XDE-007 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes”, (V.A. Linscombe, *et al.,* Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI., Report # 011071, 23 July 2001). Lymphocytes from male Sprague-Dawley rats (outbred Crl: CD BR) (stimulated for 48 hours with PHA) were exposed to XDE-007 (98.4% purity) in duplicate cultures for 4 hours, in the presence and absence of S9, at 0 (1% DMSO), 3.13, 6.25, 12.5, 25, 50, 100, and 200 *:*g/ml in the initial assay with harvest 20 hours later. In the confirmatory assay, continuous treatment for 24 hours at the same concentration levels (as the initial assay) was used in the absence of S9. Cultures were exposed 4 hours at 0, 12.5, 25, 100, and 200 *:*g/ml in the presence of S9. Cultures in both assays were harvested 24 hours after treatment initiation. Based on the mitotic indices, treatment levels 25, 100, and 200 *:*g/ml were chosen for evaluation (100 cells per replicate) of chromosomal damage. Mitotic indices indicated moderate toxicity at higher concentrations. Positive controls were functional. No increase in chromosomal aberrations. **Acceptable. (Green and Gee, 10/2//02).

DNA DAMAGE

52905-014 186506, ““Evaluation of XDE-007 in the *In Vivo* Mouse Micronucleus Assay”, (Gregory L. Erexson, Covance Laboratories, Inc., Vienna, VA., Covance Study # 22129-0-455OECD and Dow Study # 011077, 9 August 2001). 7 male Crl:CD-1®(ICR)BR mice per group received XDE-007 technical (98.4%) once daily on 2 consecutive days at 0 (0.5% methylcellulose), 500, 1000, and 2000 mg/kg/day. Use of a single sex (male) was based on a preliminary test at these same doses in 4/sex/dose. There were no mortalities due to XDE-007 in either trial. Bone marrow from 6 per group was sampled 24 hours after the last dose. For micronuclei, 2000 PCEs per animal were examined. The PCE/NCE also was determined. There was no significant decrease in PCE/NCE. No increase in micronuclei. **Acceptable. (Green and Gee, 10/3/02).

NEUROTOXICITY

Not required at this time.

SUPPLEMENTAL, INSECT METABOLISM

52905-025 186517, “Kinetics of Uptake, Clearance, Transfer, and Metabolism of Hexaflumuron

and XR-007 in Termites (*Reticulitermes flavipes)*", (J.J. Sheets, *et al.*, Dow AgroSciences LLC, Indianapolis, IN., Report # DAI0149, 2 June 1999). This assay compared the uptake, clearance, transfer, and metabolism of the radiolabelled chitin synthesis inhibitors hexaflumuron and XR-007 in termites (*Reticulitermes flavipes*).

The termite holding apparatus for the uptake kinetics phase consisted of a cylindrical plexiglass chamber (5 cm wide x 4 cm high) containing a 2:1 mixture of sand and vermiculite. Coupled to the chamber through a piece of Tygon tubing (2 mm x 10 cm) was a single feed chamber of identical dimensions containing the cellulose diet (filter paper). Cellulose was treated with [^{14}C] labeled hexaflumuron or XR-007 at 0.1% and 0.5%. Hexaflumuron and XR-007 were labeled in different rings and mixed in equal proportions (pg. 6). Radio chemical purity of all 4 samples was > 95% (pg. 7). Each apparatus contained 100 termites. The amount of radioactivity contained in each live termite was measured by scintillation counting at 2, 6, 10, 15, 20, 30, and 40 days after treatment began. At each time point, 5 groups of 5 termites each were sampled and digested with 200 :l of Protosol^{TM}. Only live termites were measured.

For clearance rate measurements, 1000 termites were placed in a petri dish and force fed cellulose disk diets containing either 0.1% or 0.5% [^{14}C] XR-007 or [^{14}C] hexaflumuron. Termites were then transferred in groups of 100 into an apparatus consisting of a single feeding chamber with untreated paper disks connected with a plastic tube to a housing chamber. 5 groups of 5 termites each were sampled a 0, 16, 30, 48, 72, 144, and 240 hours after removal from the treatment diet. Termites were placed into 4 ml plastic scintillation vials and digested in 200 :l Protosol^{TM}. Radioactivity content of the termites was determined by scintillation spectrophotometry.

For trophallaxis transfer evaluation, approximately 1000 termites were placed in each of four petri dishes (18 cm diameter) containing paper diet treated with a 0.1% acetone solution of Nile Blue, 2.6 :l/mg/dry paper and with either 0.1% or 0.5% w/w of [^{14}C] XR-007 or [^{14}C] hexaflumuron. Termites were allowed to feed on the treated paper for 7 days. The blue dyed termites were then mixed with untreated (white) termites in an apparatus consisting of a housing and feeding chamber. The feeding chamber contained untreated filter paper. Treated/untreated mixing ratios were 1:5, 1:10, and 1:20, with total number of termites per apparatus of 210. White termites (untreated) were measured (scintillation counting after Protosol^{TM} digestion) for the amount of radioactivity in their bodies at 8 hours, and at 1, 2, 4, 7, and 10 days after mixing of the 2 termite populations.

For internal dose response, toxicity was measured by placing groups of 100 termites (*R. flavipies*) into holding apparatuses having a single cellulose feeding container. Groups consisted of 4 radiolabelled concentrations of [^{14}C] XR-007 or [^{14}C] hexaflumuron, 0.00195%, 0.0065%, 0.0195%, and 0.065%, one unlabelled dose, 0.00065%, and 10 control (untreated) groups. Five replicates were done for each concentration. Mortality was recorded 15, 20, 25, 30, 45, and 60 days after initial exposure to the treated paper. At 20, 30, 40, 50, and 60 days after exposure initiation, two live termites for each apparatus were sampled for radioactivity. The grading of the observations consisted of 3 descriptions: **OK** = normal, **Affected** = moving slowly, **Collapsed** = no foraging and > 80% morality, representing 0%, 50%, and 100% mortality respectively. When the population in an apparatus was judged to have collapsed, any live termites were collected and sampled for radioactivity. The amount of XR-007 and hexaflumuron in the non-labeled group, 0.00065%, was estimated by linear regression from the labeled group data.

To evaluate termite metabolism of the test compounds, the sand/vermiculite mixtures within the housing chamber of termites fed 0.1% and 0.5% [^{14}C] XR-007 or [^{14}C] hexaflumuron for 40 days were extracted 3 times each with acetone, and filtered through a 0.45 *:*m membrane. The solvent was evaporated and the residue suspended in a small volume of acetone and spotted onto thin-layer chromatography (TLC) plates for development.

Results

XR-007 was more toxic and faster acting than hexaflumuron in all the experiments performed in this study. Internal uptake of XR-007 was generally less than hexaflumuron, especially at higher concentrations, yet the time to kill and the minimum toxic concentration were lower. Hexaflumuron was cleared from termites in a first order process with a half life of 8-9 days. The half life of XR-007 was 29 days for 0.5% and 191 days for 0.1%. Neither compound was metabolized by the termites. Both XR-007 and hexaflumuron were transferred horizontally to other termites by trophallaxis in a highly efficient manner (near 100% efficiency of transfer) during the first 8 hours of association. With increasing time, the amount of XR-007 inside of termites dropped to a steady state by about day 4 while hexaflumuron bodily concentrations continued to fall, eventually reaching zero.

Supplemental information. (Green and Gee, 10/02/02).

52905-026 186518, "Uptake Rate of [^{14}C] Hexaflumuron Force-Fed to Groups of *Reticulitermes flavipes*", (J.J. Sheets, *et al.*, Dow AgroSciences LLC, Indianapolis, IN., Report # DEI0376, 2 May 1997). Evaluation of the uptake of the [^{14}C] labeled chitin synthesis inhibitor hexaflumuron in termites (*Reticulitermes flavipes*) was performed. Results were compared with limited previous data obtained with *R. santonensis*.

The termite cage apparatus consisted of 3 cylindrical plexiglass chambers 5.0 cm in diameter, and 4.0 cm high. The center housing chamber contained a half volume mixture of sand and vermiculite (2:1 v/v), and was connected with small Tygon tubing (2 mm in diameter, 10 cm long) to 2 additional feeding chambers which contained hexaflumuron treated paper. 200 termites per apparatus were introduced into the housing chamber and allowed to forge and make contact with the connected feeding chambers. After 3-4 days of acclimation, paper disks treated with either 0.1% or 0.5% w/w [^{14}C] hexaflumuron (Lot # F0032-149, sp. act. of 30.6 mCi/mmole, > 99% purity) were placed in the feeding chambers. 10 termites from each apparatus (conducted in duplicate) were collected from the feeding chambers daily after exposure to treated paper disks and placed in scintillation vials and treated with Protosol. The amount of radioactivity contained in the termites was determined (5 minutes) by scintillation spectrophotometry. The amount of paper consumed at each time point was estimated by visual inspection. The amount of hexaflumuron and its metabolites contained in each termite was calculated based on the known specific activity of the radiolabeled material diluted with non-labeled hexaflumuron.

At both treatment levels, the amount of uptake of radioactivity initially increased rapidly with time and then leveled off at a maximum steady state concentration. The initial rate of hexaflumuron uptake (calculated for the first 7 days of the experiment) at 0.1% was 12.7 ng/termite/day with a steady state level of between 110-130 ng/termite achieved after 14-18 days of exposure. Termites force-fed the diet of 0.5% hexaflumuron had an initial uptake rate of 55 ng/termite/day with a steady state concentration of 320 ng/termite after 9-12 days.

A direct comparison of the rate of uptake of [^{14}C] hexaflumuron for the two termite species could not be made due to the difference in techniques used to measure the radioactivity. In the *R. flavipes* assay, the termites were solubilized with Protosol to eliminate any quenching of the radioactivity by the insect proteins during scintillation counting, *R. santonensis* were not. Time to maximum steady state concentration was compared and observed to be 2 times faster for *R. flavipes* versus *R. santonensis* (14-18 days versus 32 days).

No attempt was made to measure the location of hexaflumuron in or on the body of termites, rate of elimination or characterization of the radioactivity.

Supplemental information. (Green and Gee, 10/02/02).

SUBCHRONIC STUDIES

(Oral)

008; 186500; "XR-007: 4-Week Dietary Toxicity Study in Fischer Rats" (Lick, S.J. et al., Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI, Laboratory Project Study ID 971106, 10/9/97). XR-007 (Lot # DECO-615-112, purity = 99.6%) was admixed to the feed and fed to 5 Fischer 344 rats per sex per dose at dose levels of 0, 1, 10, 100, 500, or 1000 mg/kg/day (0, 1.0, 10.4, 101.4, 512.6, 1029.1 mg/kg/day, respectively for males and 0, 1.1, 10.9, 105.1, 520.6, 1055.6 mg/kg/day, respectively for females) for 4 weeks. No mortalities occurred. No treatment-related clinical signs were observed. A treatment-related increase in mean relative liver weight was observed in both sexes at 500 and 1000 mg/kg/day. Microscopic examination revealed treatment-related hepatocellular hypertrophy (centrilobular) in males at 1000 mg/kg/day and in females at 500 and 1000 mg/kg/day. **No adverse effects.** NOEL (M) = 101.4 mg/kg/day and NOEL (F) = 105.1 mg/kg/day (based on an increase in mean relative liver weight and hepatocellular hypertrophy). **Supplemental** (because only 5 animals per sex per dose were used, the animals were treated for only 4 weeks, no ophthalmological examinations were conducted, and no analysis of the dosing material was conducted). (Corlett, 10/3/02)

007; 186499; "XDE-007: 28-Day Dietary Toxicity Study in CD-1 Mice" (Yano, B.L. and Day, S.J., Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, MI, Laboratory Project Study ID 001248, 6/12/01). XDE-007 (Lot, Reference No. F0031-148, TSN102332, purity = 98.4%) was admixed to the feed and fed to 5 CD-1 mice per sex per dose at dose levels of 0, 10, 100, 500, or 1000 mg/kg/day (0, 10.8, 110, 538, 1060 mg/kg/day, respectively for males and 0, 11.2, 113, 504, 1140 mg/kg/day, respectively for females) for 28 days. No mortalities occurred. No treatment-related clinical signs were observed. Treatment-related increases in mean platelet level and mean cholesterol level were observed in both sexes at 100, 500, and 1000 mg/kg/day. A treatment-related increase in mean relative liver weight was observed at in males at 100, 500, and 1000 mg/kg/day and in females at 500 and 1000 mg/kg/day. Microscopic examination revealed treatment-related hepatocellular hypertrophy with altered tinctorial properties (centrilobular/midzonal to panlobular) in males at 500 and 1000 mg/kg/day and very slight vacuolation (consistent with fatty change) of the periportal hepatocytes in males at 500 and 1000 mg/kg/day and in females at 1000 mg/kg/day. **No adverse effects.** NOEL (M) = 10.8 mg/kg/day and NOEL (F) = 11.2 mg/kg/day (based on increases in mean platelet and mean cholesterol levels). **Supplemental** (because only 5 animals per sex per dose were used and because the animals were treated for only 28 days). (Corlett, 9/30/02)